LETTERS TO THE EDITOR

The Letters to the Editor section is subdivided into four categories entitled Communications, Notes, Comments and Errata. The textual material of each Letter is limited to 1200 words minus the following: (a) 200 words for a square figure one-column wide. Larger figures are scaled in proportion to their area. (b) 50 words for each displayed equation; (c) 7 words for each line of table including headings and horizontal rulings. Proof will be sent to authors. See the issue of 1 January 1981 for a fuller description of Letters to the Editor.

COMMUNICATIONS

Time-resolved spectroscopy of macromolecules: Effect of helical structure on the torsional dynamics of DNA and RNA[a)]

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The study of the internal motions of flexible macromolecules is of considerable interest as a probe of their structure, conformation, and mechanical properties.[1-3] Of particular interest are the naturally occurring macromolecules DNA and RNA because their mechanical properties are important in biological processes such as supercoiling and packaging in chromatin and ribosomes. In the past, internal motions have been probed by dynamic light scattering and NMR. A more direct approach to this problem has utilized the time-resolved fluorescence depolarization of ethidium bromide intercalated in DNA.[4-6] These experiments selectively probe torsional motions about the helix axis. How the torsional rigidity of DNA and RNA depends on their sequence, base pairing, and conformation is very important, but unknown.

In this communication we present new results on the torsional rigidity of a series of model polynucleotides obtained by the fluorescence depolarization technique. These compounds (Table I) were chosen on the basis of their known primary and secondary structures, and are of three types: double-stranded DNA and RNA, which differ in their helical conformations, and a DNA/RNA hybrid, which has a triple helical structure. The base sequences were chosen to illustrate the differences between A–T and G–C base pairing, and between homogeneous and alternating self-complementary sequences. Our results demonstrate that the helical structure is the dominant factor determining the torsional rigidity.

The samples contained 100 μg/ml of nucleic acid (PL Biochemicals) and 1.0 μg/ml of ethidium bromide dissolved in 0.10 M tris-HCl, *p*H 7.7/0.15 M NaCl. Time-resolved fluorescence measurements were performed with our mode-locked argon ion laser and time-correlated single photon-counting instrument, which we have described elsewhere.[4,5] The time-resolved intensities of emission polarized parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ to the polarization of the excitation pulse ($\lambda = 5145$ Å, $\Delta t = 100$ psec) were measured for each sample.[7] The width of the instrument response function (250 psec) was sufficiently narrow that the decay curves were not distorted by convolution and therefore the rotational correlation function was directly constructed as $r(t) = [I_{\parallel}(t) - I_{\perp}(t)]/[I_{\parallel}(t) + 2I_{\perp}(t)]$.

If DNA behaves as a uniformly elastic rod, then $r(t)$ obeys the following decay law[1]:

$$r(t) = r_0\left\{\tfrac{1}{4} + \tfrac{3}{4}\exp\left[-\frac{2kT}{\pi}(t/b^2\eta C)^{1/2}\right]\right\}, \qquad (1)$$

where C is the torsional rigidity, η the solvent viscosity, and b the helix radius. The parameters r_0, $b^2\eta C$, and the fluorescence lifetime τ were obtained from a nonlinear least-squares fit to the data.[4] The torsional rigidities were obtained using $b = 13.5$ Å for the double helix, obtained from hydrodynamics,[8] and $b = 14.3$ Å for the triple helix, estimated from the x-ray structure.[9] The quality of the fits, as judged by the reduced chi-squared criterion, were very good: $\chi_r^2 = 1.1$–1.4. A typical set of data is shown in Fig. 1.

The torsional rigidities of the samples in Table I exhibit interesting variations. Firstly, note that d(G–C)·d(G–), rA·rU, and calf thymus DNA have es-

TABLE I. Properties of ethidium–nucleic acid complexes.

Sample[a]	τ (nsec)	r_0	$b^2\eta C/10^{-35}$ (erg^2 sec)	$C/10^{-19}$ (erg cm)
rA·rU	27.1	0.40	2.4	1.3
d(G–C)·d(G–C)	23.3	0.37	2.6	1.4
CT	22.6	0.36	2.4	1.3
d(A–T)·d(A–T)	24.9	0.39	1.2	0.6
Denatured CT	22.6	0.40	1.2	0.6
dA·dU	22.0	0.38	0.7	0.4
dA·rU·rU	23.3	0.37	6.3	3.1

Error ranges: $\tau \pm 0.2$ nsec, $r_0 \pm 0.01$, $b^2\eta C \pm 0.3\times10^{-35}$ erg^2 sec, $C \pm 0.2\times10^{-19}$ erg cm

[a]Abbreviations: CT is calf thymus DNA; rA·rU is poly rA·poly rU, where r denotes a ribonucleotide; similarly for the other compounds

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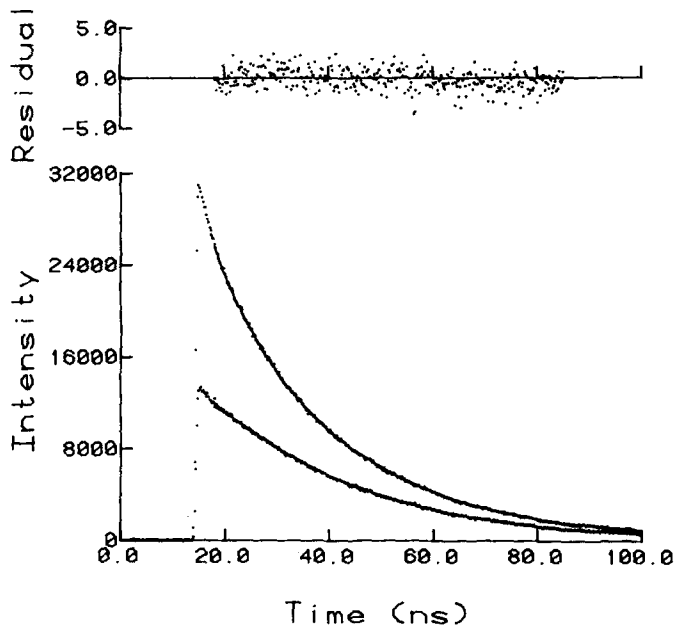

FIG. 1. Experimental $I_{\parallel}(t)$ (upper curve) and $I_{\perp}(t)$ (lower curve) for the ethidium–poly d(A–T) · poly d(A–T) complex in 0.10 M tris-HCl, pH 7.7/0.15 M NaCl. The solid lines are the best fit ($\chi_r^2 = 1.33$) of the data to the model. Also shown is the weighted residual of the fit to the combined data $I_{\parallel}(t) - I_{\perp}(t)$.

sentially equal torsional rigidities, i.e., the torsional rigidity is not sensitive to the base sequence or the helical conformation. However, d(A–T) · d(A–T) is considerably more flexible than d(G–C) · d(G–C). In fact, the torsional rigidity of d(A–T) · d(A–T) is the same as that of partially denatured calf thymus DNA. This suggests that the enhanced torsional flexibility is related to local denaturation of the double helix, a consequence of the weaker A–T hydrogen bonding. Since the average wavelength of the torsional modes contributing to the anisotropy decay is about 100 base-pair separations (corresponding to an average relaxation time of 25 nsec), the good agreement of $r(t)$ with Eq. (1) implies that on this scale d(A–T) · d(A–T) and partially denatured calf thymus DNA behave effectively as uniform rods. Therefore, the single-stranded regions must be relatively short and randomly distributed, and confer an enhanced flexibility over regions of a few hundred base pairs. The ability of the laternating self-complementary d(A–T) · d(A–T) to locally denature below the melting transition is also indicated by the formation of hairpin helix branches.[10]

The homopolymer dA · dU is even more flexible than d(A–T) · d(A–T), and does not fit Eq. (1) as well ($\chi_r^2 \geq 1.5$). Here the extent of local denaturation must be much greater, with longer single-stranded regions, so that the assumption of a uniformly elastic double helix is not as valid. It is unusual that dA · dU shows this behavior, while rA · rU does not.

The torsional rigidity of the triple-stranded[11] dA · rU · rU is considerably larger than for any of the double-stranded species ($C = 3.1 \times 10^{-19}$ erg cm). Since $C = \frac{1}{2}\mu\pi b^4$ for a uniform cylinder[12] (μ is the torsional modulus), a larger torsional rigidity is expected for the triple helix: We calculate C(triple)/C(double) = 1.3, compared with the observed value of 2.4. This result indicates that the torsional modulus of a triple helix is significantly greater than a double helix, suggesting that the triple helix is inherently less flexible.

The variation in the torsional rigidities presented in Table I are consistent with the following picture: An intact double helix has a torsional rigidity $C = 1.3 \times 10^{-19}$ erg cm, independent of the base sequence or helical conformation. A triple helix is more rigid, while the presence of single-stranded regions make the helix less rigid. Thus, *the helical structure is the dominant factor determining the torsional rigidity. Primary structure is important only insofar as it determines the helical structure*, as seen in the striking difference between d(G–C) · d(G–C) and d(A–T) · d(A–T).

In conclusion, these experiments have shown how the helical structure of nuclei acids may be probed, making it possible to obtain structural information in a variety of situations. The picosecond fluorescence depolarization technique is quite general and opens the possibility for studying conformational dynamics in a wide range of macromolecules. A full account of this work will be published later.

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